Galanin modulates oxytocin release from rat hypothalamo--neurohypophysial explant *in vitro* — the role of acute or prolonged osmotic stimulus

Galanina moduluje uwalnianie oksytocyny z układu podwzgórzowo--przysadkowego szczura *in vitro* — rola ostrego lub przewlekłego bodźca osmotycznego

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Abstract

Introduction: Galanin (Gal) may be involved as the neuromodulator of different processes in the central nervous system in the regulation of neurohypophysial function. The aim of the present study was to examine the influence of Gal on oxytocin (OT) release *in vitro*: an acute or prolonged osmotic stimulus was used as the stimulatory agent.

Material and methods: Experiments were carried out on three-month old male rats which acted as donors of isolated rat hypothalamus (Hth), neurohypophysis (NH) or hypothalamo-neurohypophysial explants (Hth-NH). The effect of Gal on OT secretion was studied under conditions of non-osmotic (i.e. K^+-evoked) (series 1), direct osmotic (i.e. Na^+-evoked) (series 2) or indirect osmotic stimulation (series 3; neural tissues were obtained from animals drinking 2% NaCl). OT content was determined by radioimmunoassay.

Results: Galanin added into incubative media caused the inhibition of basal OT release from NH and Hth-NH explants prepared from euhydrated rats but stimulated basal and K^+-stimulated OT release from the Hth tissue. Gal did not exert any influence on Na^+-evoked OT secretion. We observed increased basal OT secretion from NH and K^+-evoked respective OT release from Hth and Hth-NH explants taken from osmotically challenged rats under the influence of Gal.

Conclusions: Present experiments *in vitro* show that:

1. Galanin plays the role of an inhibitory neuromodulator of OT release from the neurohypophysis; its effect is opposite at the hypothalamic level.

2. Galanin acts as the stimulatory neuromodulator of OT release in response to prolonged osmotic stimulus; an acute osmotic stimulus blocks OT-ergic neurons susceptible to Gal. **(Endokrynol Pol 2013; 64 (2): 139–148)**

Key words: galanin, oxytocin, osmotic stimulus, in vitro explants

Streszczenie

Wstęp: Galanina (Gal) jako neuromodulator różnych procesów w ośrodkowym układzie nerwowym może być zaangażowana w regulacji funkcji części nerwowej przysadki. Celem obecnych doświadczeń było zbadanie wpływu Gal na uwalnianie oksytocyny (OT) *in vitro*: zastosowano ostry lub przewlekły bodziec osmotyczny jako czynnik pobudzający.

Materiał i metody: Badania zostały przeprowadzone na 3-miesięcznych szczurach samcach, od których pobierano podwzgórze (Hth), część nerwową przysadki (NH) lub układ podwzgórzowo-przysadkowy (Hth-NH). Wpływ Gal na uwalnianie OT był badany w warunkach braku pobudzenia osmotycznego — stymulacja jonami K^+ (seria 1), podczas ostrego pobudzenia osmotycznego wywołanego jonami Na^+ zawartymi w płynie inkubacyjnym (seria 2) lub w trakcie stymulacji osmotycznej o charakterze pośrednim, kiedy tkanki nerwowe pobierano od zwierząt otrzymujących do picia 2% roztwór NaCl (stan przewodnienia hipertonicznego) (seria 3). Zawartość OT oznaczano metodą radioimmunologiczną.

Wyniki. Seria 1: Galanina obecna w medium inkubacyjnym hamowała podstawowe uwalnianie OT z NH i układu Hth-NH, natomiast pobudzała podstawowe i stymulowane jonami K^+ uwalnianie OT z Hth. Seria 2: Gal nie wywierała żadnego wpływu na sekrecję OT indukowaną jonami Na^+ zawartymi w płynie inkubacyjnym. Seria 3: Gal była przyczyną wzrostu podstawowego uwalniania OT z NH oraz stymulowanego jonami potasu uwalniania OT z Hth oraz układu Hth-NH pobranych od szczurów w stanie przewodnienia hipertonicznego.

Wnioski. Na podstawie obecnych badań *in vitro* można przyjąć, że:

1. Gal pełni funkcję neuromodulatora hamującego uwalnianie OT z części nerwowej przysadki, natomiast wywiera efekt przeciwny na poziomie podwzgórza.

2. Gal działa jako neuromodulator pobudzający uwalnianie OT w odpowiedzi na stan przewlekłego pobudzenia osmotycznego; ostry bodziec osmotyczny blokuje wrażliwość neuronów OT-ergicznych na działanie galaniny. **(Endokrynol Pol 2013; 64 (2): 139–148)**

Słowa kluczowe: galanina, oksytocyna, bodziec osmotyczny, metodyka in vitro

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Introduction

In a similar way to vasopressin (AVP), oxytocin (OT) is produced by the neurons of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus, and after its transport towards the neurohypophysis, is released into the blood. OT is involved in the regulation of different physiological processes in females and males, such as the function of the uterus and mammary glands, cardiovascular regulation and neuroendocrine function [1]. Moreover, it has been suggested that OT plays a role as a nonhypertensive natriuretic agent. OT has been demonstrated to affect the process of natriuresis in water-deprived rats [2]. The states of hypernatraemia and plasma hyperosmolality activate magnocellular OT-ergic neurons [3, 4], resulting in increased OT release into the blood [5, 6]. A prolonged salt-loading state has been found to significantly increase OT mRNA levels [7]. In addition, OT content of the hypothalamic SON, PVN and the neurohypophysis decreases with increased OT plasma level [6, 8, 9]. Therefore, it has been postulated that a positive feedback mechanism may exist between OT secretion and plasma sodium concentration [10, 11].

Numerous neuromediators/neuromodulators are involved in regulating the activity of the hypothalamo-neurohypophysial system [12–15]. Many studies have emphasised the role of galanin (Gal) in the modulation of the release of neurohypophysial hormones [16–20]. Gal is widely distributed in the rat central nervous system (CNS) [21–23] with the highest expression in the hypothalamus, amygdaloid complex, brainstem and spinal cord as well as in the neurohypophysis [24, 25]. In the rat hypothalamus, neurons containing Gal have been observed mainly in the preoptic area, supraoptic nuclei and paraventricular nuclei [in magnocellular (mPVN) as well as parvocellular (pPVN) subdivision of PVN], in arcuate nucleus and median eminence [21, 26]. Gal coexists with AVP or OT in the neurons of SON and PVN [27, 28]. SON and PVN appear to have a high density of binding sites for all three types of Gal [29, 30]. Gal receptor type 1 (GalR1) is present mainly in the CNS, whereas large amounts of GalR2 and GalR3 are expressed at low levels in both the CNS and peripheral tissues [31].

It has been shown that Gal may exert a significant effect on the function of the hypothalamo-neuro-hypophysial (Hth-NH) system in different states of water-electrolyte balance. Some authors [16, 17] have noted that centrally-infused Gal diminishes hypertonic saline-induced AVP as well as OT secretion. Some of our earlier studies confirm the participation of Gal in OT release *in vivo* from the neurohypophysis in dehydrated, as well as osmotically challenged, rats [6,

32]. It was observed that intracerebroventricular (*icv*) injections of Gal into rats deprived of water distinctly inhibited OT release from the Hth-NH system [32]. Gal has been seen to induce similar effects after *icv* injections into salt-loaded rats i.e. the OT content of the hypothalamus and neurohypophysis rises and its level in the plasma decreases [6]. Our latest *in vitro* study [33] demonstrated the distinct inhibitory influence of Gal on basal OT release from the neurohypophysis, as well as hypothalamo-neurohypophysial explants taken from rats in a state of equilibrated water-electrolyte balance. Gal did not exert any effect on K^+-evoked OT release from either NH or Hth-NH explants. However, both basal and K^+-stimulated types of OT release from the hypothalamus were distinctly intensified under the influence of Gal [33].

Therefore, the aim of the present *in vitro* study was to investigate the possible influence of Gal on OT release from the hypothalamus, neurohypophysis or hypothalamo-neurohypophysial system in states of direct or indirect hyperosmotic stimulation.

Material and methods

Subjects

One hundred and forty three male three-month-old Wistar rats weighing 250–290 g were used in all *in vitro* experiments. They were housed four animals per cage under conditions of constant room temperature (20–22°C), humidity and lighting: a 12h/12h light/dark cycle, with lights on from 6 a.m. The animals received standard pelleted food and tap water *ad libitum*; however, in the third series of experiments, 2% NaCl solution was provided instead of tap water for eight days before decapitation. The experimental procedures were performed with the consent of the Lodz Local Commission of Ethics.

Drugs

Galanin [Gal] was purchased from Bachem [Galanin (rat) lot 0560209]. OT, for standard curve preparation as well for iodination with ^{125}I, was obtained from Peninsula Laboratories Europe Ltd.

Procedures

On the day of the experiment, the animals were decapitated between 9.00 and 10.00 a.m. The procedure of decapitation was carried out very quickly with the use of a guillotine. Each rat was decapitated in the laboratory room separately from the other animals. The brain and the pituitary with intact pituitary stalk were carefully removed from the skull: the disruption of the cranial bones has been started from the superior area. Three types of neuronal tissue were then prepared: (*i*) a block of tissue containing the hypothalamus (Hth),

or (*ii*) the neurohypophysis (NH), or (*iii*) the intact hypothalamo-neurohypophysial system (Hth-NH) [34].

The hypothalamus and the neurohypophysis were taken from the brain of the same animal, but other rats were used for isolation of the hypothalamo-neurohypophysial system. The blocks of hypothalamic tissue were dissected as follows: rostral limit — frontal plane situated about 1.0 mm anterior to the anterior margin of the optic chiasm; caudal limit — frontal plane just behind the mammilary bodies; lateral limits — sagittal planes passing, on both sides, just through the hypothalamic fissures [6, 35]. The depth of dissection was approximately 2.5–3.0 mm from the base of the brain. A single explant was approximately 5 mm wide and weighed approximately 35-40 mg. The total dissection time was about 3 min from the decapitation. This hypothalamo-neurohypophysial explant (Hth-NH) contained the suprachiasmatic nucleus (SCN) as well as the SON and PVN hypothalamic nuclei with intact axonal projections to the neurohypophysis: the anterior lobe of the pituitary having been excised [6, 36]. The hypothalamic tissue was divided into two parts before incubation [37].

Each type of isolated neural tissue was placed immediately in a polypropylene tube with 1 ml of Krebs-Ringer fluid [termed normal KRF (nKRF)] containing: 120 mM NaCl, 5 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM KH_2PO_4, 0.7 mM $MgSO_4$, 22.5 mM $NaHCO_3$, 10 mM glucose, 1.0 g/L bovine serum albumin and 0.1 g/L ascorbic acid (pH = 7.37–7.46; osmolality 280–290 mOsm/Kg H_2O). Tubes were placed in a water bath at 37°C and constantly gassed with carbogen (a mixture of 95% O_2 and 5% CO_2). At the beginning of the experiment, the appropriate type of prepared tissue was equilibrated in nKRF which was then aspirated twice and replaced with 1 ml of fresh medium. After 80 minutes of such preincubation, the nKRF was discarded and the tissues were incubated for 20 minutes in either 1 ml of nKRF alone, or containing the appropriate concentrations of galanin.

In the first series of experiments, the effect of Gal on non-osmotically stimulated (i.e. potassium-evoked) OT release was studied using neural tissues isolated from euhydrated animals. The appropriate type of prepared tissue (Hth, NH, Hth-NH) isolated from rats drinking tap water *ad libitum* (euhydrated animals) was incubated successively in: (1) Normal KRF (B1); (2) Modified KRF containing the excess of potassium ions (56 mM; NaCl concentration in the medium was appropriately reduced to maintain the osmolality) (S1); (3) The incubation fluid as (1) alone or with Gal at the concentrations of 10^{-10} or 10^{-8} M (B2); and finally (4) The KRF as (2) alone, or with Gal in the same concentrations as (3) (S2). Incubation in each medium proceeded for 20 min. In between incubation periods (2) and (3), the tissues were washed in the normal medium and these samples were discarded.

In the second series of experiments, the effect of Gal on basal and Na^+–stimulated (i.e. acute osmotic stimulation) OT release was studied. The appropriate type of prepared tissue (Hth, NH, Hth-NH) isolated from rats drinking tap water *ad libitum* (euhydrated animals) were incubated successively in (1) normal KRF (B1); (2) modified KRF containing an excess of sodium chloride (medium osmolality in the range of 320–330 mOsm/Kg H_2O) (S1); (3) the incubation fluid as (1) alone or with Gal at concentrations of 10^{-10} or 10^{-8} M (B2); and finally (4) the KRF as (2) alone or with Gal in the same concentrations as (3) (S2).

In the third series of experiments, the effect of Gal on basal and K^+-evoked OT release from the neural tissues obtained from osmotically challenged animals was studied: rats drinking 2% NaCl for eight days. The experimental protocol was the same as in the first series.

In euhydrated rats (series 1) as well as in salt-loaded rats (series 3), the plasma osmolality and haematocrit index were estimated. Plasma osmolality was measured in duplicate by freezing point depression using a semimicroosmometer (Knauer & Co GMBH, Berlin, Germany). The body weight of rats from series 3 was determined at the beginning and at the end of the eight-day period of salt loading. The initial and final body mass of the rats in the first series of experiments was similarly estimated before and after eight days of maintenance of animals under standard conditions.

After each incubation, the media were aspirated and samples immediately frozen and stored at –25°C until OT estimation by radioimmunoassay.

Radioimmunoassay (RIA)

The concentrations of OT in the medium samples were determined by double-antibody specific RIA. Anti-OT antibodies were raised in the Department of Experimental Physiology, Chair of Experimental and Clinical Physiology, Medical University of Lodz. A more detailed description of antibodies has been given earlier by Cisowska-Maciejewska and Ciosek [6]. For iodination with ^{125}I and for standard curve preparation, the chloramine-T method using standard OT (Oxytocin synth.) was used. The lower limit of detection for the assay was 1.25 pg OT/100 μL; the intra-assay coefficient of variation for OT was less than 5% (all samples within each experimental series were tested in the same RIA to avoid inter-assay variability).

Statistical evaluation of the results

OT release into incubative media was estimated by calculating the ratios between the two incubation peri-
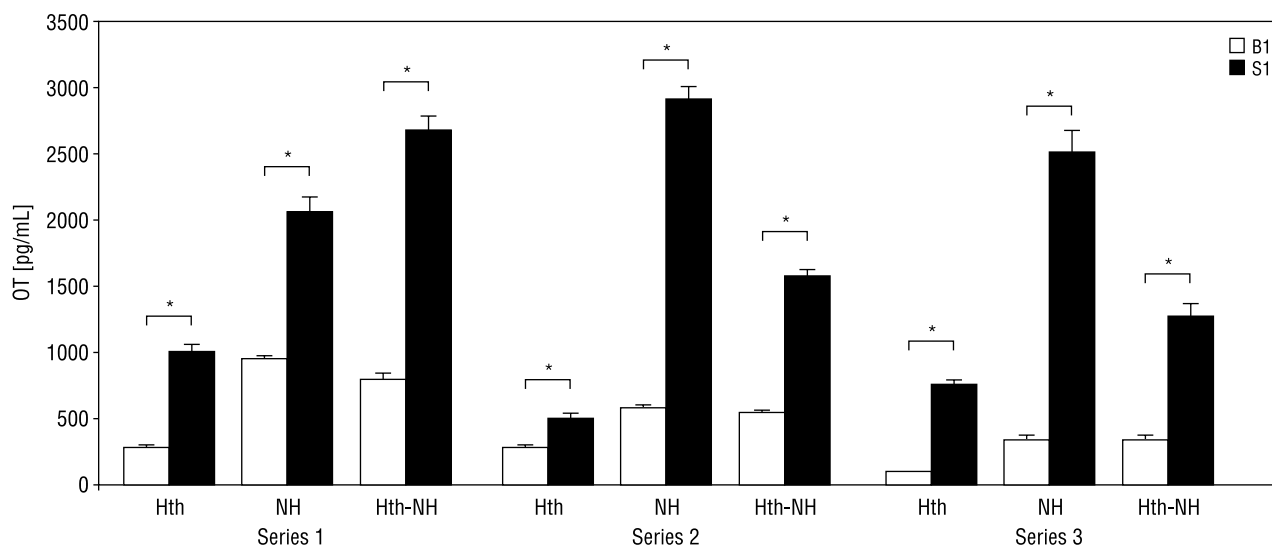
Figure 1. *Comparison of the basal (period B1) and stimulated (period S1) oxytocin (OT) release from isolated rat Hth, NH or Hth-NH explants as estimated from all experiments of each series (mean ± SEM); *p < 0.001*

Rycina 1. *Porównanie podstawowego (okres B1) i stymulowanego (okres S1) uwalniania oksytocyny (OT) z wyizolowanych Hth, NH lub układów Hth-NH oszacowane na podstawie wszystkich doświadczeń każdej serii (mean ± SEM); *p < 0.001*

Table I. *Body weight, plasma osmolality and haematocrit in rats drinking tap water or 2% saline*

Tabela I. *Masa ciała, osmolalność osocza i wskaźnik hematokrytowy u szczurów otrzymujących do picia czystą wodę lub 2-procentowy roztwór soli*

Rats	Initial body weight [g]	Final body weight [g]	Haematocrit (%)	Plasma osmolality [mOsmol/kg H_2O]
Euhydrated	272 ± 2	288 ± 3	46 ± 1	281 ± 2
Salt-loaded	272 ± 3	233 ± 3	51 ± 1	329 ± 2
Statistical significance	NS	p < 0.001	p < 0.001	p < 0.001

Values are mean ± S.E.M.

ods B2/B1 (basal release) and S2/S1 (stimulated release) for each kind of incubated tissue: the hypothalamus, neurohypophysis or hypothalamo-neurohypophysial system. The final results of OT secretion were expressed as a percentage of the control value. In each experimental group, the results were calculated and expressed as means ± S.E.M. Significance of the differences between the means was determined by the use of the Kruskal-Wallis analysis of variance (ANOVA) followed by the Mann-Whitney 'U' test (the comparison of two means); p < 0.05 was considered as the minimal level of significance.

Results

Stimulation with potassium (incubation phase S1 of series 1 and 3) or sodium (osmotic stimulus) ions (incubation phase S1 of series 2) caused augmented OT release (p < 0.001) from the isolated Hth, NH and Hth-NH explants (Fig. 1).

The values for body weight, plasma osmolality and haematocrit index in euhydrated and salt-loaded rats are shown in Table I. The body weight of rats drinking 2% saline for eight days (series 3) was reduced by 14% and their plasma osmolality and haematocrit were markedly increased (about 17% and 11%, respectively) compared to control values obtained from euhydrated rats (series 1).

Series 1. Incubation of neural tissues isolated from intact (control) rats (Fig. 2A, B, C)

Gal added into the basal medium at concentrations of 10^{-10} and 10^{-8} M diminished OT secretion from both the NH and Hth-NH explants (Fig. 2B and C: p < 0.02). However, when applied at a concentration of 10^{-10} M, Gal stimulated basal OT release during incubation of the hypothalamic tissue (Fig. 2A: p < 0.05). Although, under K^+-stimulation OT release from NH and Hth-NH complex did not change under the influence of either tested Gal concentration (Fig. 2B and C), OT secretion from the hypothalamus was seen to rise (Fig. 2A: p < 0.02 and p < 0.05).
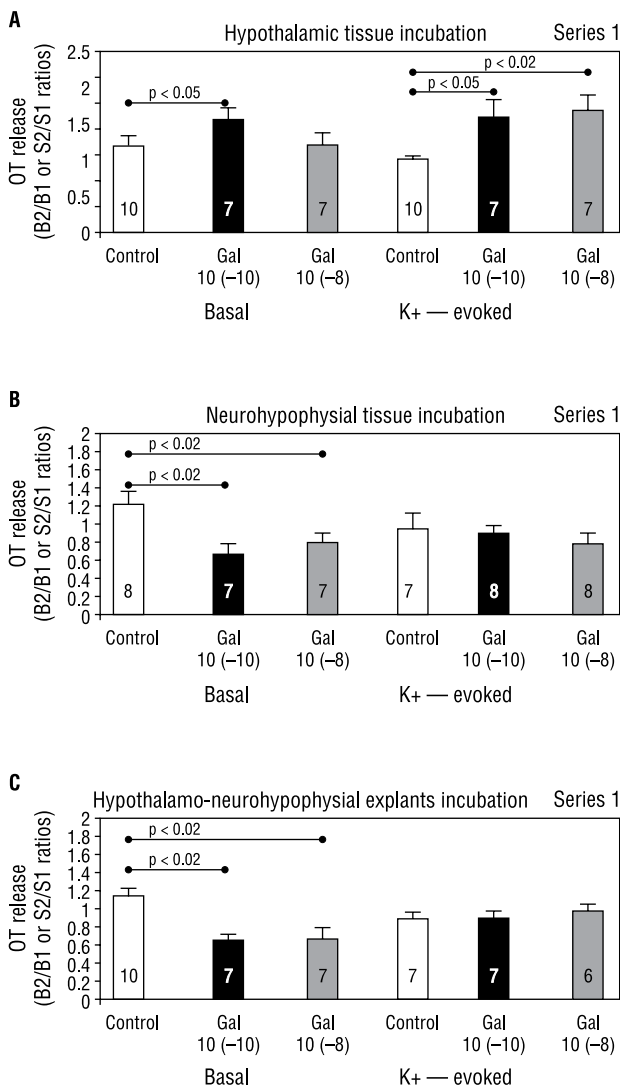
Figure 2A. *The effect of galanin (Gal) on the basal and K+-evoked OT release from the hypothalamus obtained from euhydrated rats (series 1). Each bar represents mean ± SEM and figures in bars indicate the number of samples per group.* **B.** *The effect of galanin (Gal) on the basal and K+-evoked OT release from the neurohypophysis obtained from euhydrated rats (series 1). Each bar represents mean ± SEM and figures in bars indicate the number of samples per group.* **C.** *The effect of galanin (Gal) on the basal and K+-evoked OT release from the hypothalamo-neurohypophysial explants obtained from euhydrated rats (series 1). Each bar represents mean ± SEM and figures in bars indicate the number of samples per group*

Rycina 2A. *Wpływ galaniny (Gal) na podstawowe oraz indukowane jonami K+ uwalnianie OT z podwzgórza izolowanego od szczurów w stanie równowagi wodno-elektrolitowej (seria 1). Wyniki przedstawiają średnią ± SEM; dane w kolumnach określają liczbę próbek w grupie.* **B.** *Wpływ galaniny (Gal) na podstawowe oraz indukowane jonami K+ uwalnianie OT z części nerwowej przysadki izolowanej od szczurów w stanie równowagi wodno-elektrolitowej (seria 1). Wyniki przedstawiają średnią ± SEM; dane w kolumnach określają liczbę próbek w grupie.* **C.** *Wpływ galaniny (Gal) na podstawowe oraz indukowane jonami K+ uwalnianie OT z układu podwzgórzowo-przysadkowego izolowanego od szczurów w stanie równowagi wodno-elektrolitowej (seria 1). Wyniki przedstawiają średnią ± SEM; dane w kolumnach określają liczbę próbek w grupie*

Series 2. Incubation of neural tissues exposed to direct osmotic stimulation (i.e. evoked by Na+ ions added into the medium) (Fig. 3A, B, C)

During the basal period of the incubation in Krebs-Ringer fluid, Gal action was similar to that observed in series 1, OT secretion was inhibited from NH and Hth-NH explants at both Gal concentrations, while it was raised from Hth tissue at 10^{-10} M Gal (Fig. 3A: $p < 0.05$; Fig. 3B and C: $p < 0.02$ and $p < 0.05$). When neural tissues were incubated in the medium enriched in Na+ ions (acute osmotic stimulus), Gal did not exert a significant influence on OT secretion (Fig. 3A, B and C).

Series 3. Incubation of neural tissues isolated from osmotically challenged animals (Fig. 4A, B, C)

Gal at a concentration of 10^{-10} M markedly increased basal OT secretion from NH (Fig. 4B: $p < 0.02$) while 10^{-10} and 10^{-8} M Gal also increased potassium-stimulated OT release from Hth and Hth-NH explants (Fig. 4A and C: $p < 0.01$, $p < 0.02$ and $p < 0.05$). Gal did not modify potassium-evoked OT secretion from the NH (Fig. 4B).

Discussion

These results demonstrate the distinct role played by Gal in the release of OT from the hypothalamo-neurohypophysial system *in vitro*. These results clearly show that Gal's action depends on the different states of water-electrolyte balance in the experimental animals, as well as on the electrolyte composition of the incubation fluid.

In fact, the release of the neurohormones of posterior lobe of the pituitary is closely connected with the actual state of water-electrolyte homeostasis [6, 38]. Both AVP-ergic and OT-ergic neuron activities, as well as AVP and OT mRNA expression, increase significantly in the rat hypothalamus in response to acute or chronic osmotic stimuli such as salt-loading or water deprivation [39, 40]. The respective information is sent to some brain structures involved in osmoregulatory mechanisms related to AVP-ergic as well as OT-ergic neurons excitation [41–43]. Therefore, the number of Fos immunoreactive neurons increases in such hypothalamic osmosensitive structures as the medial preoptic area (MnPO), organum vasculosum of the lamina terminalis (OVLT), PVN and SON after *icv* osmotic stimulations [43]. It is worth noting that OT secretion from the posterior pituitary in response to salt-loading is even greater than AVP release [44–46]. In the present study, two modes of OT-ergic neuron stimulation have been applied, i.e. non-osmotic (potassium-evoked) and osmotic stimuli (sodium ions-evoked). Osmotic stimulation of OT-ergic neurons may have a direct character, brought about by an excess of sodium ions added into the incubative fluid, or an
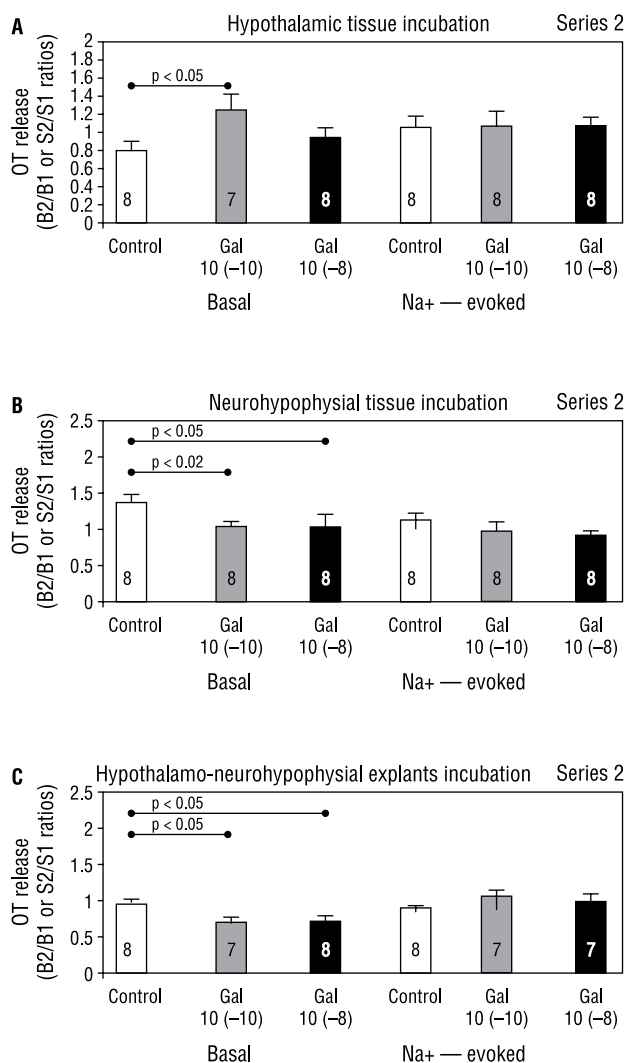
Figure 3A. *The effect of galanin (Gal) on the basal and Na^+-evoked OT release from the hypothalamus obtained from euhydrated rats (series 2). Each bar represents mean ± SEM and figures in bars indicate the number of samples per group.* **B.** *The effect of galanin (Gal) on the basal and Na^+-evoked OT release from the neurohypophysis obtained from euhydrated rats (series 2). Each bar represents mean ± SEM and figures in bars indicate the number of samples per group.* **C.** *The effect of galanin (Gal) on the basal and Na^+-evoked OT release from the hypothalamo-neurohypophysial explants obtained from euhydrated rats (series 2). Each bar represents mean ± SEM and figures in bars indicate the number of samples per group*

Rycina 3A. *Wpływ galaniny (Gal) na podstawowe oraz indukowane jonami Na^+ uwalnianie OT z podwzgórza izolowanego od szczurów w stanie równowagi wodno-elektrolitowej (seria 2). Wyniki przedstawiają średnią ± SEM; dane w kolumnach określają liczbę próbek w grupie.* **B.** *Wpływ galaniny (Gal) na podstawowe oraz indukowane jonami Na^+ uwalnianie OT z części nerwowej przysadki izolowanej od szczurów w stanie równowagi wodno-elektrolitowej (seria 2). Wyniki przedstawiają średnią ± SEM; dane w kolumnach określają liczbę próbek w grupie.* **C.** *Wpływ galaniny (Gal) na podstawowe oraz indukowane jonami Na^+ uwalnianie OT z układu podwzgórzowo-przysadkowego izolowanego od szczurów w stanie równowagi wodno-elektrolitowej (seria 2). Wyniki przedstawiają średnią ± SEM; dane w kolumnach określają liczbę próbek w grupie*
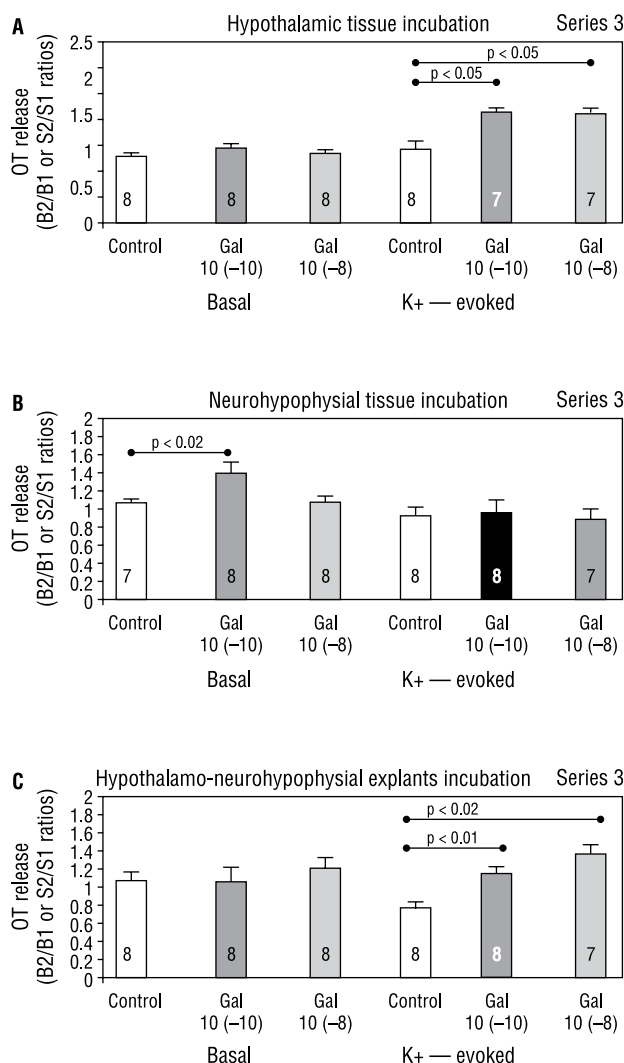
Figure 4A. *The effect of galanin (Gal) on the basal and K^+-evoked OT release from the hypothalamus obtained from rats drinking 2% saline for eight days (series 3). Each bar represents mean ± SEM and figures in bars indicate the number of samples per group.* **B.** *The effect of galanin (Gal) on the basal and K^+-evoked OT release from the neurohypophysis obtained from rats drinking 2% saline for eight days (series 3). Each bar represents mean ± SEM and figures in bars indicate the number of samples per group.* **C.** *The effect of galanin (Gal) on the basal and K^+-evoked OT release from the hypothalamo-neurohypophysial explants obtained from rats drinking 2% saline for eight days (series 3). Each bar represents mean ± SEM and figures in bars indicate the number of samples per group*

Rycina 4A. *Wpływ galaniny (Gal) na podstawowe oraz indukowane jonami K^+ uwalnianie OT z podwzgórza izolowanego od szczurów otrzymujących do picia 2% roztwór soli przez 8 dni (seria 3). Wyniki przedstawiają średnią ± SEM; dane w kolumnach określają liczbę próbek w grupie.* **B.** *Wpływ galaniny (Gal) na podstawowe oraz indukowane jonami K^+ uwalnianie OT z części nerwowej przysadki izolowanej od szczurów otrzymujących do picia 2% roztwór soli przez 8 dni (seria 3). Wyniki przedstawiają średnią ± SEM; dane w kolumnach określają liczbę próbek w grupie.* **C.** *Wpływ galaniny (Gal) na podstawowe oraz indukowane jonami K^+ uwalnianie OT z układu podwzgórzowo-przysadkowego izolowanego od szczurów otrzymujących do picia 2% roztwór soli przez 8 dni (seria 3). Wyniki przedstawiają średnią ± SEM; dane w kolumnach określają liczbę próbek w grupie*

indirect character, connected with afferent impulsation originating from systemic and central osmoreceptors in salt-loading rats [5, 41, 47, 48].

The addition of sodium ions to incubative media causes direct osmotic excitation of magnocellular neurons. This leads to membrane depolarisation of AVP-ergic and OT-ergic neurons, thus increasing their firing rate and the release of both neurohormones release [41]. Under *in vitro* conditions, it is mainly the perikarya of these neurons which react to direct osmotic stimulation [49], whereas potassium-evoked depolarisation excites the cell bodies of these neurons and the terminals of their axons in the neurohypophysis.

A prepared neurohypophysis incubated *in vitro* is without doubt the classic and most convenient model for studying AVP-ergic and OT-ergic neuron activity, and has been used for many years [36, 50]. These tissues maintain the ability to modulate their AVP and OT secretion in response to stimuli for at least a few hours [12, 51]. It has been assumed that in this incubation model, the changes in neurohormone release are due to the direct action of various biologically active compounds on neurosecretory endings in the posterior lobe of the pituitary.

For the *in vitro* study of AVP and OT release, the whole isolated hypothalamo-neurohypophysial system is also used [51, 52]. This model enables the neural consecutiveness of the hypothalamo-neurohypophysial tracts to be maintained, which ensures the integrality of the processes taking place there. It has been assumed that most nervous fibres ending by the synapses in the AVP-ergic and OT-ergic bodies within the paraventricular and supraoptic nuclei of the hypothalamus are intact. In such a prepared specimen, the permanence of neural hypothalamo-neurohypophysial tracts is maintained, as are most processes related to the biosynthesis, transport, and secretion of AVP and OT [49].

Based on earlier reports [53], a prepared block of neural tissue including the hypothalamus and the part of the thalamus which contained the neurons of paraventricular and supraoptic nuclei as well as suprachiasmatic nuclei, was used in the present study. This approach allows the hypothalamic AVP-ergic and OT-ergic neurons incubated in the medium to be exposed directly to the tested compounds. The observed changes of AVP and OT secretion into the incubated fluid allow the biosynthetic rate of both neurohormones, as well as their release into the surrounding tissues, to be estimated at the hypothalamic level.

Hypothalamo-neurohypophysial system activity may be modulated by the participation of many different neuromodulators/neuromediators in the central nervous system (CNS), one of which is galanin. Gal-like neurons, Gal receptors (GalRs) as well as Gal

mRNA have been observed in the hypothalamus, median eminence and posterior lobe of the pituitary [21]. Some authors [19, 30] have noted the presence of the receptors for Gal (GalR1, GalR2, GalR3) in the MnPO, PVN and SON. GalR1 mRNA has been shown to exist in high amounts in SON, mPVN and pPVN by *in situ* hybridisation studies [19, 21]. GalR2 mRNA has been detected in the PVN but not in SON [21]. Gal-ergic neurons from different areas of the brain project to areas of the hypothalamus, including SON and PVN, as well as the anteroventral region of the third ventricle (AV3V) [26, 42]. Rich Gal-ergic projection innervates AVP-ergic and OT-ergic neurons in hypothalamic magnocellular nuclei. High Gal immunoreactivity has been observed in the perinuclear area of magnocellular neurons and in their dendrites [21, 54–56]. Colocalisation of Gal with OT is possible, and has been demonstrated in such experimental conditions as treatment with colchicin, an alkaloid inhibiting neurohormonal axonal transport [57, 58] or hypophysectomy [8, 59]. High expression of Gal mRNA is found in the PVN and SON in the presence of OT [8, 60].

Our earlier *in vivo* and *in vitro* studies demonstrate the possible influence of Gal on OT and/or AVP release. AVP release was found to be inhibited when Gal was injected centrally to hypertonic saline-treated rats [16]. No effect of *icv* injection of Gal on OT mRNA content has been found in dehydrated rats [61]. On the other hand, although Björkstrand et al. [17] observed a significant decrease of OT plasma level in rats after *icv* injection of Gal, this has yet to be confirmed by other studies [62]. Our team observed *icv* injections of galanin to have an inhibitory influence on OT secretion in dehydrated animals, as well as those drinking hypertonic saline [6, 32]. Our previous studies also note that Gal has an inhibitory effect on basal OT release from NH and Hth-NH explants *in vitro,* when different concentrations are added into incubative media [33]. Our most recent study demonstrates that Gal added into incubative media inhibits basal (but not potassium-stimulated) AVP release from the Hth-NH complex of osmotically challenged animals [34].

The present results of control incubation (series 1) indicate that Gal may play a neuromodulatory role in the secretion of OT from the hypothalamo-neurohypophysial system under *in vitro* conditions, but this effect is undirected. The inhibitory effect of Gal on basal OT release from incubated NH and Hth-NH explants confirms the data presented above [33]. These results are also in agreement with earlier *in vitro* experiments by Gálfi et al. [63] who observed concentrations of Gal ranging from 10^{-6} to 10^{-9} M to have an inhibitory influence on basal OT release from the 13–14 day neurohypophysial cell cultures.

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However, in the present study, Gal was seen to intensify OT secretion from the hypothalamic tissue both in basal and K$^+$-evoked conditions, which corresponds with the results obtained in our earlier studies [33]. Moreover, it is worthy of note that only the lowermost Gal concentration (10^{-10} M) exerted such an effect: higher concentrations displayed no significant influence on OT release from incubated hypothalamic tissue.

On the other hand, the contradictory nature of Gal on OT secretion confirms our previous observations: its stimulatory influence on Hth contrasts with its inhibitory effect on other incubated tissues (NH or Hth-NH complex) [33]. It cannot be excluded that the cell bodies of OT-ergic neurons can respond to Gal in a different manner to the axonal endings of these neurons in the neurohypophysis. Moreover, there is the high probability that Gal can inhibit the infundibular transport of both neurohormones from the hypothalamus towards the neurohypophysis. Such action of Gal may lead to decreased OT release from the posterior lobe of the pituitary as well as from the hypothalamoneurohypophysial system.

In the second series of tests, acute osmotic stimulus induced by an excess of sodium ions present in the medium was seen to block the influence of Gal on OT release. No change in Na$^+$-stimulated OT release was found, however, basal OT secretion under Gal influence had the same character as in series 1. This effect is difficult to interpret, but it could be caused by the disturbed sensibility of Gal receptors to this peptide during acute osmotic stimulation.

The third series of tests in the present study used salt-loaded rats as the donor animals of neural tissues for *in vitro* incubation. It is known that hyperosmolality of extracellular fluids, such as plasma and CSF, is the main stimulus for AVP and OT release from the neurohypophysis into the blood. Experimental animals drinking a hypertonic solution of sodium chloride for a few days show a prompt increase of plasma osmolality, as well as AVP and OT release [6, 64, 65]. In the present *in vitro* experiments, OT plasma level was not determined in salt-loading rats after decapitation. However, it is known from earlier studies that OT concentration in the blood of animals receiving hypertonic solution to drink rises significantly [6, 66]. In the present study, the third series of rats received a 2% solution of NaCl *ad libitum* for eight days before decapitation. This concentration of saline has been used in previous studies as it is known to provoke hypertonic overhydration [6, 65], as confirmed by the significant increases of plasma osmolality and haematocrit index values seen in salt-loaded animals in series 3.

In the present study, the OT-ergic neurons of the hypothalamo-neurohypophysial system obtained from osmotically challenged rats responded to Gal added into the medium in a different way than during acute osmotic stimulus. Although Gal stimulated basal OT secretion from NH and K$^+$-evoked OT release from the Hth and Hth-NH explants, it did not change basal OT release from the Hth and Hth-NH system. It is hard to comment on this significant difference between the reactions of OT-ergic neurons to Gal under the influence of either acute or chronic osmotic stimuli. However, the possibility that Gal receptors adapt to a prolonged state of osmoreceptors activation cannot be ruled out. Also, this present observation does not confirm earlier findings that Gal injected *icv* into salt-loaded rats diminishes OT secretion from the neurohypophysis into the blood [6]. However, Burazin et al. [67] noted increased levels of Gal-R1 mRNA as well as the intensity of Gal-R1-like immunostaining in the SON and magnocellular PVN after four days of salt-loading, and propose that Gal may influence AVP synthesis and/or release (OT as well) *via* autocrine or paracrine activation of Gal-R1 receptors especially during long-lasting stimulation.

However, it cannot be excluded that the effects of such regulation of neurohypophysial neurohormone release may be different under *in vivo* or *in vitro* conditions. Moreover, the distorted Gal receptor function in salt-loaded rats may be the reason for the different responses seen when Gal is incubated with either neural tissue or neural fragments taken from intact animals (series 1 and 2). Gundlach and Burazin [68] suggest that a differential expression (or level of expression) of Gal receptor subtypes exists in different hypothalamic and other neuronal areas, as well as the possibility of a differential response of these receptors in situations where the amount of galanin peptide is increased.

Papas and Bourque [69] and Depczyński et al. [70] postulate that the hyperpolarising properties of Gal for the membrane activity of magnocellular neurons restrict AVP and OT release from neurohypophysial axonal terminals. Kozoriz et al. [71] demonstrated not only that Gal presynaptically reduces eEPSCs (excitatory postsynaptic currents) of SON magnocellular neurons (MCNs) in non dehydrated and dehydrated rats, but also that Gal increases the postsynaptic hyperpolarisation of MCNs after two days of dehydration. It is possible that Gal reduces depolarisation of MCNs and then restricts AVP and OT release. Our present results related to OT release *in vitro* under the influence of Gal in different water-electrolyte states seem to only partially agree with these studies.

Conclusions

Our *in vitro* experiments show that:
1. Gal plays the role of an inhibitory neuromodulator of OT release from the neurohypophysis; its effect is opposite at the hypothalamic level.

2. It may be assumed that Gal acts as the stimulatory neuromodulator of OT release in response to prolonged osmotic stimulus; an acute osmotic stimulus blocks OT-ergic neurons susceptible to Gal.

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